



## *Streptococcus agalactiae* mastitis: A review

Gregory P. Keefe

**Abstract** — *Streptococcus agalactiae* continues to be a major cause of subclinical mastitis in dairy cattle and a source of economic loss for the industry. Veterinarians are often asked to provide information on herd level control and eradication of *S. agalactiae* mastitis. This review collects and collates relevant publications on the subject. The literature search was conducted in 1993 on the Agricola database. Articles related to *S. agalactiae* epidemiology, pathogen identification techniques, milk quality consequences, and control, prevention, and therapy were included.

*Streptococcus agalactiae* is an obligate parasite of the bovine mammary gland and is susceptible to treatment with a variety of antibiotics. Despite this fact, where state or provincial census data are available, herd prevalence levels range from 11% (Alberta, 1991) to 47% (Vermont, 1985). Infection with *S. agalactiae* is associated with elevated somatic cell count and total bacteria count and a decrease in the quantity and quality of milk products produced. Bulk tank milk culture has, using traditional milk culture techniques, had a low sensitivity for identifying *S. agalactiae* at the herd level. New culture methods, using selective media and large inocula, have substantially improved the sensitivity of bulk tank culture. Efficacy of therapy on individual cows remains high. Protocols for therapy of all infected animals in a herd are generally successful in eradicating the pathogen from the herd, especially if they are followed up with good udder hygiene techniques.

**Résumé** — **Mammite à *Streptococcus agalactiae* : analyse documentaire.** Le *Streptococcus agalactiae* continue d'être une cause importante de mammite subcliniques dans les troupeaux laitiers et de pertes économiques pour l'industrie. On demande souvent aux vétérinaires de fournir des informations sur le contrôle et l'éradication des mammite à *S. agalactiae* dans les troupeaux. Cette analyse recueille et rassemble les publications pertinentes. L'analyse documentaire a été faite en 1993 en utilisant la banque de données Agricola. Les articles reliés à l'épidémiologie de *S. agalactiae*, aux techniques d'identification du pathogène, aux conséquences sur la qualité du lait ainsi que ceux sur le contrôle, la prévention et la thérapie ont été retenus.

Le *Streptococcus agalactiae* est un parasite obligatoire de la glande mammaire des bovins et est susceptible d'être combattu par plusieurs antibiotiques. En dépit de ces constatations, dans tous les états ou les provinces où des relevés épidémiologiques sont disponibles, le taux de prévalence au niveau des troupeaux varie de 11 % (Alberta, 1991) à 47 % (Vermont, 1985). L'infection à *S. agalactiae* est associée à un comptage élevé de cellules somatiques et de bactéries totales et à une diminution de la quantité et de la qualité des produits laitiers. La culture du lait provenant de réservoir de conservation en vrac selon les techniques traditionnelles avait une faible sensibilité pour identifier le *S. agalactiae* au niveau du troupeau. De nouvelles méthodes de culture, utilisant des milieux sélectifs et des inocula massifs, ont grandement amélioré la sensibilité des méthodes de culture à partir des réservoirs en vrac. Les protocoles de thérapie de tous les individus infectés d'un troupeau sont en général efficaces pour éradiquer le pathogène d'un troupeau, spécialement s'ils sont accompagnés de bonnes techniques d'hygiène du pis.

(Traduit par docteur André Blouin)

Can Vet J 1997; 38: 429-437

### Introduction

**M**astitis is a major source of economic loss to the dairy industry (1), and milk quality and the prevalence of mastitis are major factors in determining farm

profitability (2). Despite the fact that a sustained extension and educational effort has been ongoing since the early 1970s, mastitis remains the single health factor that is most influential in affecting milk production, with both clinical and subclinical mastitis having a major effect on milk yield (3). Based on responses to a questionnaire sent to dairy personnel in each state, the annual cost of mastitis in the United States was estimated at 1.3 billion dollars

Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island C1A 4P3.

in 1976 (4). This represented 11% of total farm receipts for milk sales, or \$117.35 per cow (4). Other studies have estimated the annual milk loss due to mastitis caused by the major contagious pathogens, *Streptococcus agalactiae* and *Staphylococcus aureus*, at 10% (5).

Veterinarians play an important role in the management of mastitis. They are increasingly relied upon for information related to herd management of animal health, as well as individual animal diseases. Dairy herd managers ranked mastitis as the most important disease on their farms (6). These farmers consulted veterinarians more frequently than any other professional group about this problem. It is important to note, however, that 16 of 42 producers sought advice from someone other than a veterinarian, such as a processor's field representative, county extension agent, or feed company representative, as their primary source of mastitis information (6). It is evident that if veterinarians are to remain in a leadership position, relative to the provision of udder health services to dairy producers, they must continue to upgrade their knowledge and skills (7).

## Materials and methods

The literature search for this paper was conducted on the Agricola database. Key words used in the search included *Streptococcus agalactiae*, group B streptococci, subclinical mastitis, and contagious mastitis. The search was conducted in 1993 and included all articles published since 1980. Publications prior to 1980 deemed to be of historical significance were also included. All papers identified through the Agricola search containing relevant and unique information pertinent to the topic were included. Additional publications dated after the search period have been added during preparation of the manuscript. Most studies that are cited incorporated good statistical design, and where sampling of populations was used, random sampling techniques were employed. Studies that used nonrandom or convenience sampling techniques were included, if they added pertinent information. Studies that used convenience sampling methods are identified as such in the text.

## Results

### Overview of *Streptococcus agalactiae*

*Streptococcus agalactiae* was a major cause of mastitis in the pre-antibiotic era. It remains a significant cause of chronic mastitis in many herds, even though it can be readily eliminated (8). Procedures for the diagnosis and treatment of intramammary infections due to the bacterium are well established (9). Since it can survive for long periods only within the mammary gland and is susceptible to penicillin therapy, eradication within a closed herd is possible (10). Herds can be maintained free of infection with *S. agalactiae* under field conditions (11).

*Streptococcus agalactiae* has the ability to adhere to the mammary tissue of cows and the specific micro-environment of the bovine udder is necessary for the growth of the bacterium (12). The virulence of various strains is related to differences in their ability to adhere to the mammary epithelium (8).

*Streptococcus agalactiae* also causes neonatal septicemia in humans. Human infection is generally acquired from other human sources, although there may be some risk associated with direct exposure to infected animals or their products. Overall there is considerable homology between strains isolated from septicemic infants and mastitic cows (13). Although some surface antigens seem to be specific to the cow (14), *Streptococcus agalactiae* is now recognized to be part of the normal bacterial flora in the human throat, genitourinary tract, and rectum (15).

### Epidemiology

*Streptococcus agalactiae* is a highly contagious obligate parasite of the bovine mammary gland (10). It generally causes a low-grade persistent type of infection and does not have a high self-cure rate (16). Unidentified infected cattle function as reservoirs of infection, because they are not selected for treatment, segregation, or culling (17). For an obligate intramammary pathogen like *S. agalactiae*, the bovine udder is recognized as the only reasonable source of the organism in the milk. Consequently, isolates in the bulk tank are usually assumed to have come from the udder (18,19).

When a herd is infected with *S. agalactiae*, traditionally there has been a high within-herd prevalence (19). In 1982, an average of 39.5% of cows were infected within positive herds in Mississippi (20). In Massachusetts, an intraherd prevalence of 44.7% was found among infected herds between 1976 and 1982 (21). More recent studies in 1990 (22) and 1992 (23) showed a trend toward lower within-herd prevalences, with individual cow infection levels in positive herds averaging 7.9% and 10%, respectively. In the 1992 study, the authors noted that infection status had a marked positive skew, with most herds having a very low proportion of quarters infected and a few herds having a high proportion. This is in sharp contrast to the Massachusetts study (21), in which *S. agalactiae*-positive herds had major pathogens isolated from 58.5% of cows, with 69% of these isolates being *S. agalactiae*.

### Herd prevalence studies

Prevalence studies have been conducted in a number of areas. Some studies were based on the culture of milk samples from individual cows, while others were based on the culture of bulk tank milk samples. All figures quoted below are of herd level prevalence. *Streptococcus agalactiae* was isolated from either the bulk tank milk or the culture of at least 1 individual cow sample in the herd. Some of the prevalence data cited in the review were based on census of the entire study population, while others were based on a sample, random or convenience, of the population.

### Bulk tank cultures

In a 1982 census of herds in Mississippi ( $n = 998$ ), 435 herds (44%) were positive for *S. agalactiae* (20). In a census of the 2931 herds shipping milk in Vermont in 1985, *S. agalactiae* was isolated on a single, bulk tank milk culture from 47% of herds (24). In 1990, the study was repeated on 1971 of these herds that were still in production. The herd prevalence was found to be 32%

(25). In a census of herds in Alberta in 1991 ( $n = 1350$ ), the herd prevalence of infection was found to be 11% (26). In a census of herds in Prince Edward Island in 1992 ( $n = 460$ ), the herd prevalence of *S. agalactiae* was found to be 18.9% (27). In a random sample of dairy herds in southwestern Ontario in 1990, 42.4% of 250 herds were found to be positive (22). In a stratified random sample of herds in the St. Hyacinthe agricultural region of Quebec in 1991, 43% of 400 herds were found to be positive (28).

In California, 44% of herds in a nonrandomly selected group ( $n = 50$ ) were found to be infected, based on a single, bulk tank milk sample (19). In an Irish study, 38.5% of 379 herds tested in a monthly bulk tank culture program were positive during the yearlong study (29). In a study of herds selling milk to a cooperative giving premiums for low somatic cell count (SCC), the herd prevalence of *S. agalactiae* was 5%, based on 802 bulk tank cultures (11).

### Individual cow samples

In a stratified random sample of herds in Ohio, using cultures of individual cow samples, 56% of 48 herds had at least 1 positive culture (23). In a separate stratified random sample study in the same state, also using individual cow samples, 34.7% of 49 herds were found to be positive (18).

In Pennsylvania, 60% of 29 nonrandomly selected herds had at least 1 cow infected with *S. agalactiae* (30). In California (31), 47 of 50 herds in a nonrandomly selected group had at least 1 cow infected with *S. agalactiae* (herd prevalence 94%). *Streptococcus agalactiae* was the most prevalent bacterium from individual cow cultures. Of the 23 138 individual cows tested, using composite milk culture, 7.81% were positive. In a review of 19 000 herd surveys conducted by the New York State Quality Milk Promotion Service, more than half had at least 1 cow infected with *S. agalactiae* (32).

It is evident from these figures that *S. agalactiae* remains an important problem in the North American dairy industry. These values represent serious economic losses to the industry. It is encouraging to note that in the study of herds shipping milk to a dairy processor paying quality premiums, the level of infection seems drastically reduced over that of other studies in the same state. This suggests that the problem can be controlled at the field level.

### Pathogen identification

If eradication programs for *S. agalactiae* are to be effective, methods for identification of the pathogen at both the herd and individual cow level need to be inexpensive, accurate, and nonlabor intensive. Researchers continue to work on the advancement of diagnostic tests both through refinements of the traditional bacteriological techniques and in the area of diagnostic immunology.

### Microbiology of *Streptococcus agalactiae*

*Streptococcus agalactiae*, a group B streptococcus, is a gram-positive coccus, often noted growing in chains in milk and in liquid media. Pasteur, Koch, and Neisser, the 1st proponents of the germ theory of disease, recognized

the role of streptococci in disease (33). In 1889, Nocard and Mollereau isolated "*Streptococcus nocardii*," later renamed *Streptococcus agalactiae*, in the original investigation of the cause of bovine mastitis. Prior to the widespread use of penicillin, researchers stated that 90% of all bovine mastitis was caused by streptococci (34).

Group B streptococci produce an extracellular product, which, in the presence of staphylococcal  $\beta$  hemolysin, causes a zone of complete hemolysis in blood agar, referred to as the CAMP reaction (named for the discoverers of the phenomenon Christie, Atkins, and Munch-Peterson) (35). Traditionally, the CAMP reaction has been read by streaking a  $\beta$ -hemolytic staphylococcus perpendicular to streaks of suspect streptococci. As early as 1968,  $\beta$ -hemolysin was extracted from staphylococci (36). The toxin was then incorporated into media to allow the direct observation of the CAMP reaction on primary plates. In the same year, other researchers, noted that the CAMP reaction observed using a crude  $\beta$ -hemolysin, which they streaked on the surface of the plates, was identical to that observed using streaks of living  $\beta$ -staphylococci (37). Others have used drops of the  $\beta$ -hemolysin to observe the CAMP reaction (38).  $\beta$  hemolysin is not commercially available, but details on its production have been published (36).

Unlike most other species of streptococci, *S. agalactiae* does not hydrolyze esculin (33). The addition of ferric citrate to media as an iron source augments the dark color production by esculin-splitting bacteria, facilitating differentiation of these bacteria from group B streptococci (33).

Another feature of group B streptococci, which has been exploited for identification purposes, is its ability to produce pigmented colonies when grown anaerobically on starch-containing media (39). Pigment production is very much dependent on anaerobic conditions, inclusion of starch in the medium (0.1%), and the pH of the medium ( $>7.3$ ) (39). The pigment has the characteristics of a carotenoid localized in the membranous portion of the cell wall (40). A close linkage has been proposed between the genes associated with pigment production and hemolysis (39). Production of the pigment appears to be more consistent among human than bovine strains (15,41).

Fresh or frozen milk samples have been used successfully in the culture of both pooled bulk tank and individual cow samples for *S. agalactiae* (17,42–44). Generally, freezing has had no effect on the recovery of *S. agalactiae* from the milk of infected cows (9,43,44). However, in 1 report, freezing increased the number of isolations (17). The viability of *S. agalactiae* in milk was determined by quantifying the number of colony-forming units before and after freezing (45). Storage at  $-20^{\circ}\text{C}$  for 4 wk did not affect the number of colonies recovered.

### Culture from bulk tank samples

Bacteria in bulk tank milk can originate from 1 of 2 sources. Bacteria can be present within the cow's udder or environmental contamination can occur as milk is removed from the cow, handled, and processed (46). Due to the obligate nature of *S. agalactiae*, its presence in bulk milk is the exclusive result of shedding of bacteria

from infected quarters (47). Consequently, the assumption of 100% specificity on bulk tank milk samples is reasonable (18). *Streptococcus agalactiae* is usually shed in high numbers from infected glands (43,48), with a cyclic shedding pattern being typical (49). The number of *S. agalactiae* in bulk tank milk is a function of the number of infected quarters shedding the organism. In a study of 7 herds, *S. agalactiae* did not multiply within the milking system or in the tank, except at temperatures higher than 27°C (19).

Although the specificity of culture of bulk tank milk can be assumed to be very high, the sensitivity has been variable among studies. Not all researchers have used the same methods. Consequently, it is difficult to compare test characteristics among the various protocols. Variations in the sensitivity of culture of bulk tank milk are probably a function of differences in the protocols, variation in the intraherd prevalence, and variations in the rate of bacterial shedding related to the stage of infection (19).

In a study of 49 herds, culture results from samples of bulk tank milk and the milk filter were compared with individual quarter milk samples (18). The method used was to streak an 0.01 mL aliquot on a quadrant of sheep blood esculin agar and trypticase soy, crystal violet, and thallium acetate (TKT) medium. The prevalence of *S. agalactiae*, based on at least 1 culture-positive cow in the herd, was 35%. The sensitivity of culture of bulk tank milk was 35% (6/17). The specificity of the test was 97% (31/32). In the same study, the use of the milk filter was found to have a sensitivity of 24% (4/17).

In a study in which composite cow and bulk tank milk samples were taken from 23 138 cows in 50 herds, 1 loopful (0.01 mL) of milk from the composite samples or samples of bulk tank milk was streaked on a half or a full blood agar plate, respectively (19). A streak of  $\beta$ -hemolysin was placed on the medium, so the CAMP reaction could be read directly. The sensitivity of cultures from bulk tank milk, using individual cow sampling as the gold standard, was 50%. Forty-six percent of the observed variation in the intraherd prevalence could be explained on the basis of the number of colonies isolated per millilitre of bulk tank, milk. In positive herds in which *S. agalactiae* was not isolated from the bulk tank, an average of 3.9% of the cows were infected. In herds in which it was isolated from the bulk tank an average of 18% of cows were infected (19).

The use of TKT medium with ferric citrate and a 0.01 mL inoculum on samples of bulk tank milk yielded a sensitivity of only 20.5% when compared with the gold standard of cultures from individual cows (22).

Schoonderwoerd *et al* (26) employed 2 selective media in a technique for the isolation of *S. agalactiae*. The 1st was a starch medium, modified to make it more selective, which was incubated under anaerobic conditions so that pigment production could be observed. The 2nd was a CAMP/esculin medium. When this technique was employed on bulk tank milk samples, a large inoculum was used and the results of the cultures were interpreted in parallel (26). Interpreting diagnostic tests in parallel reduces the false negative classification rate (50). The sensitivity of this protocol was reported to be 95% and the specificity 100% (26). Using the same

technique in a separate study, the sensitivity of a single culture of bulk tank milk was estimated to be between 65% and 78% (51).

### Culture from individual cow samples

A mastitis monitoring program based on sampling of cows at first milking, when cases of clinical mastitis occur, and at dry off has been suggested (52). Others have stated that whole herd culture gives a reliable picture of the level of contagious mastitis pathogens (16). Each of these protocols assumes that the culture of samples from individual cows is accurate. It is difficult to define the accuracy of culture of individual cow samples, as they are often the gold standard against which other culture methods are measured. Published studies measure the agreement among tests or measure the effect, if any, of various enrichment procedures.

Because no gold standard was available, Jasper *et al* (53) attempted to establish the ability of bacterial culture to identify *S. agalactiae*-infected quarters by measuring the level of agreement between duplicate samples. One quadrant of a TKT/FC plate was inoculated using 0.01 mL of milk. One hundred and seventy-three samples were eventually identified as positive. One hundred and seventy-two were identified on the initial sample.

A study involving 167 cows on 4 farms with a cow prevalence of *S. agalactiae* between 25% and 65% was carried out to determine the effects of several different sampling and cultural protocols on the number of cows diagnosed as positive (9). Quarter and composite samples containing 0.01 mL of milk were streaked on a half plate of trypticase soy blood agar with 0.1% of esculin added. Alternatively, 0.05 mL of a composite sample were inoculated on a whole plate. No differences were noted for recovery rates from milk taken before, immediately after, or 5 h after milking. The number of cows identified as positive did not increase by quarter sampling rather than composite sampling. The use of a larger volume of inoculum with composite samples did not affect the disease classification. The sensitivity and specificity of a single culture, based on infection history, ranged between 95% and 100%.

The effects of augmented culture techniques on the recovery of bacteria from cases of clinical mastitis have been evaluated (43). Results from cultures on blood agar incubated for 48 h at 37°C were compared with those in which preculture freezing, preculture incubation, and increased inoculum sizes were used. Other species of bacteria responded by increasing growth and recovery rates. However, for *S. agalactiae*, there was no effect. The authors speculated that this was because, when present, *S. agalactiae* is usually shed in high numbers (43). One study found a positive effect of pre-enrichment in a brain heart infusion (BHI) broth on the recovery rate of *S. agalactiae* compared with plating of 0.01 mL directly on blood agar (54). Incubation of composite milk samples in BHI for 6 h prior to inoculation of the blood agar plates resulted in an increase in the percentage of cows classified as infected from 6.2 to 10.8.

In the absence of a gold standard based upon a different biological principle, it is difficult to assess the sensitivity and specificity of cultures from individual cows. However, the level of bacterial shedding, the inability of

enhanced culture procedures to increase recovery in most instances, and the fact that the test is highly repeatable all indicate that the sensitivity and specificity of the test are high.

#### Other tests for *S. agalactiae* mastitis

There is a need for rapid, accurate, screening tests for the identification of *S. agalactiae* in milk samples from both bulk tanks and individual cows. Unfortunately, preliminary culture must occur before the current tests can be used. The most used of these tests is latex agglutination. When used on isolates of samples from bulk tank milk, the sensitivity and specificity of latex agglutination were 97.6% and 98.2%, respectively (55). In other studies, latex agglutination was 100% sensitive (56–58) and had a specificity of between 98% and 100% (57,58). Other tests for *S. agalactiae* include the Phadebact slide coagglutination method (59), as well as the ELISA and IFA techniques (60).

#### Milk quality

The profitability of the dairy industry is driven by both the quantity and the quality of the milk produced. Although milk has often been described in the popular media as “nature’s most perfect food,” it cannot escape consumer scrutiny for quality and wholesomeness. As a result, many milk marketing and processing organizations have begun to penalize poor quality and give financial rewards for superior quality milk. In the first 10 y of a bonus payment scheme based on bulk tank somatic cell count (BTSCC) and bacteriological criteria, producers received \$5 000 000 in bonus payments (61). The data show a steady increase in the quality of milk during that time period, suggesting that farmers do respond to bonus payment schemes.

#### Milk products

*Streptococcus agalactiae* infection in dairy cattle plays an important role in reducing the production of quality milk and milk products. Milk from cows with subclinical mastitis decreases the quality of cheese and other manufactured milk products (62). Changes in milk composition result in reduced nutritional value of milk, increased processing problems, and off flavors (63). The shelf life of fluid milk products is also decreased, due to augmentation of the growth of spoilage bacteria (63).

#### Somatic cell count

Development of techniques for rapid measurement of the SCC has contributed greatly to the advancement of mastitis control programs. Cell counting provided tangible evidence to producers of the existence of subclinical mastitis (64). The economic impact of an elevated SCC as a surrogate measure of subclinical mastitis has been reviewed elsewhere (2,4,65).

High BTSCCs have been shown to be correlated with poor milk quality, reduced quantity and quality of processed milk products, and shorter shelf life (2) and are used by the dairy industry as a measure of raw milk quality (2,65). Decreasing BTSCCs has been associated with a parallel decrease in antibiotic residue violations (2). Others noted a similar relationship among antibiotic residue violations, elevated cell counts, and *S. agalactiae*-infection

status (29). In a study of 1032 farms, the highest average SCC was found for farms with *S. agalactiae* in their bulk tank milk (66). *Streptococcus agalactiae* produces high SCC in individual cows, which has a significant influence on the BTSCC. In a group of herds with BTSCCs greater than 700 000, the geometric mean SCC for *S. agalactiae*-infected cows was 2 238 700 (67). In another study, the arithmetic mean SCC for *S. agalactiae*-infected cows was 900 000 (68). In herds with a BTSCC greater than 800 000, 80% of cows with a greater than 500 000 SCC were infected with *S. agalactiae* (69). In a study of 12 herds (70), blitz therapy for *S. agalactiae* dropped the average herd SCC from 918 000 to 439 000 in 30 d and, with the implementation of postmilking teat dip (PMTD) and dry cow therapy (DCT), to 268 000 in 1 y.

In Vermont, a drop in the herd prevalence of *S. agalactiae* from 47% to 32% over a period of 5 y corresponded with a drop in the average BTSCC for the state from  $539 \times 10^3$  cells/mL to  $337 \times 10^3$  cells/mL (25).

In a randomly selected group of herds with an average SCC greater than  $700 \times 10^3$  cells/mL, all herds had cows infected with *S. agalactiae* and 26% of quarters were infected (67). In herds with an average SCC less than  $150 \times 10^3$  cells/mL, the percentage of quarters infected with *S. agalactiae* was 0.1%.

#### Standard plate count

The total bacterial count in bulk tank milk can be substantially increased by the presence of *S. agalactiae* mastitis in a dairy herd (24,48,71). Samples of bulk tank milk from infected herds frequently contain bacterial counts in the range of 20 000 to 100 000 colony forming units (cfu) per mL (47), because a cow in the early stages of infection with *S. agalactiae* can shed up to  $100 \times 10^6$  bacteria per mL (48). The standard plate count (SPC) dropped from 99 000 to 2000 after the implementation of a modified, blitz therapy regimen and hygiene practices to control *S. agalactiae* (72).

#### Udder hygiene and therapy

Since the early 1970s when veterinarians began to analyze health problems at the herd level, the focus of mastitis control programs has shifted from the treatment of individual cases of mastitis to the development of methods for the prevention, control, and herd level therapy of mastitis. Premilking udder hygiene and good cow husbandry are essential parts of a quality milk program. Protocols for routine udder hygiene have been reviewed elsewhere (73).

#### Control and prevention

A number of factors have been found to be associated with the prevalence of *S. agalactiae* in a herd. Foremost among these has been the failure to use PMTD and the selective or nonuse of DCT (11,22,32). The use of a common wash rag or sponge has also been found to be a risk factor (23,32), as has the cleanliness of the cows, cleanliness of the exercise area, and the herd size (23). Inadequate treatment of clinical cases of mastitis was observed more frequently in herds that were infected (11). Larger herd size and nonparticipation in the Dairy Herd Improvement Association program were associated

with the disease (74). In contrast, Bartlett *et al* (23) found that there was no association between PMTD and DCT and the *S. agalactiae*-infection status of herds. These researchers speculated that this was because the practices were widely adopted by both infected and noninfected herds.

Some authors have stated that, if applied in a conscientious program, PMTD in combination with DCT will eliminate *S. agalactiae* from herds (10,64). It is possible that the widespread adoption of PMTD and DCT may have led to a drop in the intraherd prevalence from the high levels noted in the 1970s and early 1980s, but not to complete eradication. Smith and Ward (75) speculated that this would happen as early as 1975. What does seem certain is that failure to use these 2 procedures to follow up a blitz therapy eradication program can lead to considerable frustration and expense on the part of dairy producers, due to re-emergence of infection within the herd (1,76).

### Therapy

The therapeutic and preventative effectiveness of antimicrobial drugs for bovine mastitis is dependent upon the etiological agent, proper use of the drug under consideration, dairy husbandry, sanitation procedures, and the phase of the disease (77). When drugs are being selected for the treatment of subclinical mastitis, selection should be based on cost, safety, residue potential, distribution properties, and sensitivity data (78).

*Streptococcus agalactiae* is generally sensitive to intramammary therapy, using a variety of commercially available preparations. Systemic therapy has also been reported to be effective, but offers no clear medical or economic benefits over intramammary therapy (78). Treatment of *S. agalactiae* mastitis with intramammary infusion products will result in a high percentage of infections being eliminated in a cost effective manner and with few residue concerns, provided that milk withholding times are observed (79). In herds with high prevalence of *S. agalactiae*, the use of these products in cases of both clinical and subclinical mastitis is justified, because it stops bacterial shedding. It should be noted, however, that the treatment of clinical cases is not effective in reducing the herd prevalence, unless it is part of a total control program (80).

Many studies have looked at the *in vitro* activity of various antimicrobials against *S. agalactiae*. In 1 study, none of 39 strains of *S. agalactiae* from 6 states were resistant to penicillin and 95% were susceptible to lincomycin and erythromycin; 75% were susceptible to tetracyclines, but susceptibility to streptomycin and spectinomycin was much lower (81). In 2 studies, all *S. agalactiae* ( $n = 7$ ) (82) and ( $n = 14$ ) (83) were sensitive to penicillin or its derivatives, tetracyclines, and erythromycin. In Quebec, 68 isolates of *S. agalactiae* were found to be sensitive to penicillin or its derivatives and erythromycin; 1 isolate was resistant to tetracycline (84).

Sensitivity testing, *in vitro*, is an attempt to predict the likely activity of the material *in vivo* (82), but the *in vivo* behavior of the product may be different (83). So, it is important to examine some reports of activity within the mammary gland. Craven (85) reviewed 11 articles in

which the bacteriologic cure rate for *S. agalactiae* was reported following penicillin therapy. The mean cure rate was 84%. Tyler noted that both lactational and dry cow therapy results in greater than 90% cure rates (78). Huber (77) reported that 98% ( $n = 578$ ) of infected cows responded to treatment with penicillin-containing drycow therapy products and that the sensitivity to penicillin in 1977 was about the same as it was when penicillin was first introduced.

Therapy with cloxacillin eliminated the bacteria from 98% (76) and 100% (86) of cows infected with *S. agalactiae*, respectively. In a study of farms with long standing udder health problems, all cows shedding *S. agalactiae* were treated with a combined penicillin and novobiocin intramammary infusion product (70); a cure rate of 92.6% for quarters and 88.3% for cows was attained in 30 d.

In California (87), 220 cows were identified by composite milk culture as being infected and were randomly assigned to 1 of 2 treatment groups. One group was treated with a commercially available intramammary infusion product containing 100 000 IU of penicillin and 150 mg novobiocin; the other group was given  $1.2 \times 10^6$  IU of procaine penicillin G in 10 mL of sterile saline. When the infected cows were cultured again, 21 to 25 d after treatment, 90% were no longer shedding the bacterium. The cure rate with the commercial product (94%) was higher than that recorded with the home prepared penicillin suspension (87%). The previous SCC, as estimated by the California mastitis test (CMT), was an important factor affecting the treatment outcome, with high CMT cows less likely to have the infection eliminated. Age was also a factor, with heifers having higher recovery rates than older cows. Herd of origin was also associated with the outcome of therapy (87).

The management procedure that can most easily alter the percentage of quarters infected in a herd without destroying saleable milk is DCT (1). Drycow therapy generally enhances the cure rate of existing infections and should be used in herds with contagious mastitis pathogens (88). Only 36% to 42% of quarters ( $n = 212$ ) spontaneously eliminated infections due to *S. agalactiae* during the dry period. The use of DCT was associated with a quarter cure rate of 95% ( $n = 1004$ ) during the same period (89).

### *Streptococcus agalactiae* eradication programs

There are occasions when it may be beneficial to try to reduce the prevalence of subclinical mastitis in a dairy herd more rapidly than can be achieved with DCT and PMTD (1).

*Streptococcus agalactiae* is the only pathogen causing subclinical mastitis that can be treated economically during lactation (90). It was eliminated from herds that were blitz treated with antibiotics followed by good sanitation procedures more quickly than from blitz-treated herds that did not have follow up sanitation (75). Cows that are nonresponsive to the 1st treatment and are not identified for further treatment or culling can serve as reservoirs of infection. In herds where teat dipping and other hygienic practices are not adequately performed, the bacteria can quickly spread to the non-infected cows (90).

There are several reports on eradication programs and their cost effectiveness. In a study of 12 herds, lactational therapy followed by a program of PMTD and blanket DCT yielded a net benefit of 512 kg of milk and 14 kg of fat per cow in the 1st year (70). The ensuing increased milk production resulted in a cost benefit ratio of 2.28:1. Culture and treatment of positive animals had a better cost benefit ratio than did either treatment over a certain SCC threshold or total blitz-therapy of all lactating cows (70).

Ninety-seven of 99 *S. agalactiae*-infected cows in a herd ( $n = 627$ ) were found to be free of the infection after treatment with penicillin or a penicillin and novobiocin combination (49). Expected lactation curves of the untreated infected cows were compared with the lactation curves of treated infected cows and the lactation curves of a series of matched control cows. The evaluation revealed no significant difference between the milk production of infected cows posttreatment and that of cows that had never been infected. The level of production returned to normal after treatment during the same lactation. The economic benefits of the mastitis treatment program were also calculated. The simulation model predicted depressed lactation yields of approximately 25%, 16%, and 8% over the 305-day lactation period for cows becoming infected at 14, 63, or 126 d in milk, respectively. Consequently, the benefits of treatment differed with the stage of lactation. Treatment of infected cows early in lactation ( $<60$  d) yielded a net benefit of \$396 and therapy of cows in mid-lactation (61–120 d) yielded a net benefit of \$237. Treatment of cows with *S. agalactiae* in late lactation was associated with a net loss of \$55 per cow. In this latter group, the increase in production was not maintained long enough to offset the costs associated with treatment. The overall benefit to cost ratio for the blitz therapy program was 2.25 to 1 (49).

*Streptococcus agalactiae* is the most common contagious pathogen causing herd level infection in St. Croix, US Virgin Islands. In a random survey of cows (91), 96.5% were infected with a pathogen, including 26% with *S. agalactiae*. None of the producers were teat dipping or using complete DCT and most were using a common wash rag to clean cows. The authors estimated that implementing a control program could return \$317 to \$999 per cow per year.

On *S. agalactiae*-infected farms where blitz therapy of all infected cows is not possible, treatment protocols have been modified. A modified blitz-therapy program was used as part of a herd program to eliminate *S. agalactiae* from a seasonal herd. A basic udder health management program was instituted and the herd was divided into 2, based on a SCC threshold of 500 000. Those cows in the high SCC category were treated with 300 mg of erythromycin, intramammary. When lactating cow numbers ebbed to their lowest point, all animals were treated with the same product. Cows were treated at dry off with 500 mg of cloxacillin and 250 mg of ampicillin. When the herd was in full production again, all cows were cultured. None of the animals present in the herd during the treatment protocol were still positive. The program was found to have a benefit cost ratio of 1.41:1 (92). In another modification of the

blitz-therapy protocol, all cows with clinical mastitis or a CMT in any quarter greater than 2 were medicated. This therapy was combined with improvements in milking equipment and sanitation. In a case report using this method, the herd SCC dropped from 1 600 000 to 250 000 (72).

Management programs for subclinical mastitis in general and *S. agalactiae* in particular are effective in controlling herd infection. The economics of such programs are generally very favorable when response is measured by changes in the prevalence of herd infection, incidence of clinical disease, or SCC.

### National programs

Some countries have undertaken national or regional programs aimed at the eradication of *S. agalactiae*. These programs have legislative authority to implement control measures and have been successful in dramatically lowering the prevalence of the disease.

Israel has a system of regional laboratories with regulatory authority to implement a *S. agalactiae* eradication program. At 1 regional laboratory, a decrease in herd prevalence of *S. agalactiae* from 28% of herds to less than 2% was noted in the first 5 y of the scheme. Each farm had its bulk tank milk cultured monthly, and in infected herds, milk from all the lactating cows was examined individually. All cows found to be positive were treated. Cows were resampled and animals that were still positive after the 1st treatment were either culled or treated again. Less than 3% of infected cows needed to be culled because they were refractory to treatment (93).

In Denmark, herd milk supplies are cultured annually for the presence of *S. agalactiae*. All cows on farms found to be positive on bulk tank culture are sampled individually (94). Herd prevalence of the disease decreased from 15% to 2% between the 1950s and 1970s after implementation of this program.

## Conclusions

*Streptococcus agalactiae* continues to be a prevalent cause of subclinical mastitis in the North American dairy industry. With increased use of udder health technologies, such as, TDCT and PMTD, there has been a shift from high intraherd prevalence in the 1970s and early 1980s to lower intraherd prevalence in the 1990s. Subclinical mastitis associated with *S. agalactiae* can have a substantial impact on the quantity and quality of milk produced. Herd level control and eradication programs have been shown to be cost effective. There is continuing need to enhance diagnostic capability to improve sensitivity at the herd level, so that infected herds can be notified of their status in a timely manner. Provision of such information, combined with producer education programs, may reduce prevalence to levels at which regulatory programs may be initiated.

## Acknowledgments

I thank Drs. Ian Dohoo and Ken Leslie for their support.

CVJ



## References

1. Erskine RJ. Mastitis control in dairy herds with high prevalence of subclinical mastitis. *Compend Contin Educ Pract Vet* 1992; 14: 969-979.
2. Reneau JK, Packard VS. Monitoring mastitis, milk quality and economic losses in dairy fields. *Dairy Food Environ Sanit* 1991; 11: 4-11.
3. Reneau JK. Effective use of dairy herd improvement somatic cell counts in mastitis control. *J Dairy Sci* 1986; 69: 1708-1720.
4. Blosser TH. Economic losses from and the National Research Program on mastitis in the United States. *J Dairy Sci* 1979; 62: 119-127.
5. Janzen JJ. Economic losses resulting from mastitis. A review. *J Dairy Sci* 1970; 53: 1151-1161.
6. Goodger WJ, Rupanner R. Why the dairy industry does not make greater use of veterinarians. *J Am Vet Med Assoc* 1982; 181: 706-710.
7. Wise JK. U.S. market for food animal veterinary medical services. *J Am Vet Med Assoc* 1987; 190: 1530-1533.
8. Jain NC. Common mammary pathogens and factors in infection and mastitis. *J Dairy Sci* 1979; 62: 128-134.
9. Dinsmore RP, English PB, Gonzalez RN, Sears PM, Schulte HF. Evaluation of methods for the diagnosis of *Streptococcus agalactiae* intramammary infections in dairy cattle. *J Dairy Sci* 1991; 74: 1521-1526.
10. McDonald JS. Streptococcal and Staphylococcal mastitis. *J Am Vet Med Assoc* 1977; 170: 1157-1159.
11. Sischo WM, Heider LE, Miller GY, Moore DA. Prevalence of contagious pathogens of bovine mastitis and use of mastitis control practices. *J Am Vet Med Assoc* 1993; 202: 595-600.
12. Wanger AR, Dunny GM. Specific agglutination of *Streptococcus agalactiae* from bovine mastitis by casein components of bovine milk. *J Dairy Sci* 1984; 67: 2441-2445.
13. Wanger AR, Dunny GM. Development of a system for genetic and molecular analysis of *Streptococcus agalactiae*. *Res Vet Sci* 1985; 38: 202-208.
14. Wanger AR, Dunny GM. Identification of a *Streptococcus agalactiae* protein antigen associated with bovine mastitis isolates. *Infect Immun* 1987; 55: 1170-1175.
15. Mhalu FS. Infection with *Streptococcus agalactiae* in a London hospital. *J Clin Pathol* 1976; 29: 309-312.
16. Farnsworth RJ. Indications of contagious and environmental mastitis pathogens in a dairy herd. *Proc 26th Annu Meet Natl Mastitis Council* 1987; 26: 151-155.
17. Villanueva MR, Tyler JW, Thurmond MC. Recovery of *Streptococcus agalactiae* and *Staphylococcus aureus* from fresh and frozen bovine milk. *J Am Vet Med Assoc* 1991; 198: 1398-1400.
18. Bartlett PC, Miller GY, Lance SE, Heider LE. Use of bulk tank and milk filter cultures in screening for *Streptococcus agalactiae* and coagulase-positive Staphylococci. *J Food Protect* 1991; 54: 848-851.
19. Gonzalez RN, Jasper DE, Bushnell RB, Farver TB. Relationship between mastitis pathogen numbers in bulk tank milk and bovine udder infections in California dairy herds. *J Am Vet Med Assoc* 1986; 189: 442-445.
20. Sears PM, Fetting M, Marsh-Salin J. Prevalence of *Streptococcus agalactiae* from bulk tank samples and estimated economic impact. *3rd Int Symp Vet Epidemiol Econ*, Arlington, Virginia, 1982: 670.
21. Oliver SP, Mitchell BA. Prevalence of mastitis pathogens in herds participating in a mastitis control program. *J Dairy Sci* 1984; 67: 2436-2440.
22. Godkin MA, Leslie KE. The relationships between bulk tank milk culture, management factors used in mastitis control and the herd prevalence of mastitis. *Int Symp Bovine Mastitis*, Indianapolis, Indiana, 1990; 368-374.
23. Bartlett PC, Miller GY, Lance SE, Hancock DD, Heider LE. Managerial risk factors of intramammary infection with *Streptococcus agalactiae* in dairy herds in Ohio. *Am J Vet Res* 1992; 53: 1715-1721.
24. Hogan JS, Pankey JW, Murdough P, Howard DB. Survey of bulk tank milk using blood-esculin agar counts. *J Food Protect* 1986; 49: 990-993.
25. Goldberg JJ, Pankey JW, Drechsler PA, Murdough PA, Howard DB. An update survey of bulk tank milk quality in Vermont. *J Food Protect* 1991; 54: 549-553.
26. Schoonderwoerd M, Rawluk S, Ollis G, Schipper C. Prevalence of *Streptococcus agalactiae* in Alberta dairy herds. *Farming for the Future* 91.0845; Alberta Agriculture, Food and Rural Development, 1993.
27. Keefe GP, Dohoo IR. Herd prevalence and incidence of *S. agalactiae* in Prince Edward Island, Canada and evaluation of an eradication extension program. *3rd Int Mastitis Symp*, Tel Aviv, Israel, 1995; s4: 82-84.
28. Guillemette JM, Bouchard E, Bigras-Poulin M, Nadeau M. Étude sur la prevalence de *Streptococcus agalactiae* et *Staphylococcus aureus* dans les troupeaux du Québec par la culture séquentielle du réservoir. *Proc Am Assoc Bovine Pract — World Assoc Buiatrics* 1992; 3: 377-382.
29. Greer DO, Pearson JKL. *Streptococcus agalactiae* in dairy herds. Its incidence and relationship to cell count and inhibitory substance levels in bulk tank milk. *Br Vet J* 1973; 129: 544-553.
30. Eberhart RJ, Hutchinson LJ, Spencer SB. Relationships of bulk tank somatic cell counts to prevalence of intramammary infection and to indices of herd production. *J Food Protect* 1982; 45: 1125-1128.
31. Gonzalez RN, Jasper DE, Farver TB, Bushnell RB, Franti CE. Prevalence of udder infections and mastitis in 50 California dairy herds. *J Am Vet Med Assoc* 1988; 193: 323-328.
32. Dargent-Molina P, Scarlett J, Pollock RVH, Erb HN, Sears P. Herd-level risk factors for *Staphylococcus aureus* and *Streptococcus agalactiae* intramammary infections. *Prev Vet Med* 1988; 6: 127-142.
33. Koneman EW, Allen SD, Dowell VR Jr, Janda WM, Sommers HM, Winn WC Jr. *Color Atlas and Textbook of Diagnostic Microbiology*. 3rd ed. Philadelphia: JB Lippincott, 1988.
34. Schalm OW, Carroll EJ, Nain NC. *Bovine Mastitis*. Philadelphia: Lea & Febiger, 1971.
35. Brown J, Farnsworth R, Wannamaker LW, Johnson DW. CAMP factor of group B streptococci: Production, assay and neutralization by sera from immunized rabbits and experimentally infected cows. *Infect Immun* 1974; 9: 377-383.
36. Ward GE, Postle DS. Preparation and titration of crude staphylococcal *Beta* hemolysin for use in T.K.T. medium. *J Milk Food Technol* 1969; 31: 171-173.
37. Jasper DE, Dellinger JD. Use of a crude  $\beta$ -staphylococcal hemolysin for the presumptive recognition of *Streptococcus agalactiae*. *Am J Vet Clin Pathol* 1968; 2: 43-47.
38. National Mastitis Council, Inc. *Microbiological Procedures for the Diagnosis of Bovine Udder Infection*. 3rd ed. Arlington, Virginia: National Mastitis Council, 1990.
39. Fallon RJ. The rapid recognition of Lancefield group B haemolytic streptococci. *J Clin Pathol* 1974; 27: 902-905.
40. De La Rosa M, Villareal R, Vega D, Miranda C, Martinezbrocal A. Granada medium for detection and identification of group B streptococci. *J Clin Microbiol* 1983; 18: 779-785.
41. Merritt K, Jacobs NJ. Characterization and incidence of pigment production by human clinical group B streptococci. *J Clin Microbiol* 1978; 8: 105-107.
42. Hueston WD, Heider LE, Harvey WR, Smith KL. The use of high somatic cell count prevalence in epidemiologic investigations of mastitis control practices. *Prev Vet Med* 1987; 4: 447-461.
43. Dinsmore RP, English PB, Gonzales RN, Sears PM. Use of augmented cultural techniques in the diagnosis of the bacterial cause of clinical bovine mastitis. *J Dairy Sci* 1992; 75: 2706-2712.
44. Schukken YH, Smit JAH, Grommers FJ, VanDegeer D, Brand A. Effect of freezing on bacteriologic culturing of mastitis milk samples. *J Dairy Sci* 1989; 72: 1900-1906.
45. Pankey JW, Wadsworth JK, Metha KH, Murdough PA. Effects of storage on viability of mastitis pathogens. *J Dairy Sci* 1987; 70(suppl 1): 132.
46. Fuhrmann T. Practical applications of bulk tank milk analysis. *Compend Contin Educ Pract Vet* 1986; 8: s274-s280.
47. Hogan JS, Smith KL. Using bulk tank milk cultures in a dairy practice. *Nat Mastitis Council Mastitis Microbiol Diagn Workshop*, Arlington, Virginia, 1992.
48. Guterbock WM, Blackmer PE. Veterinary interpretation of bulk-tank milk. *Vet Clin North Am Large Anim Pract* 1984; 6: 257-268.
49. Yamagata M, Goodger WJ, Weaver L, Franti C. The economic benefit of treating subclinical *Streptococcus agalactiae* mastitis in lactating cows. *J Am Vet Med Assoc* 1987; 191: 1556-1561.



50. Martin SW, Meek AH, Willeberg P. Veterinary epidemiology principles and methods. Ames, Iowa: Iowa State Univ Pr, 1987.
51. Keefe GP. Herd prevalence and incidence of *S. agalactiae* in Prince Edward Island and evaluation of the effectiveness of an eradication extension program. MSc Thesis, University of Prince Edward Island, 1995.
52. Pankey JW, Hogan JS, Murdough PA. Bacteriological monitor for mastitis. Proc 26th Annu Meet Natl Mastitis Counc 1987; 26: 10-17.
53. Jasper DE, Dellinger JD, Bushnell RR. Agreement of duplicate samples of milk for the evaluation of quarter infection. Am J Vet Res 1974; 35: 1371-1373.
54. Thurmond MC, Tyler JW, Luiz DM, Holmberg CA, Picanso JP. The effect of pre-enrichment on recovery of *Streptococcus agalactiae*, *Staphylococcus aureus* and *Mycoplasma* from bovine milk. Epidemiol Infect 1989; 103: 465-474.
55. Hogan JS, Smith KL. Sensitivity and specificity of latex agglutination tests used to identify *Streptococcus agalactiae* and *Staphylococcus aureus* isolated from bulk tank milk. Am J Vet Res 1988; 49: 1537-1539.
56. Poutrel B. Comparative evaluation of commercial latex agglutination and coagglutination reagents for groups B, C, and D mastitis streptococci. Am J Vet Res 1983; 44: 490-492.
57. Watts JL, Owens WE. Evaluation of the rapid mastitis test for identification of *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine mammary glands. J Clin Microbiol 1988; 26: 672-674.
58. Facklam RR, Cooksey RC, Wortham EC. Evaluation of commercial latex agglutination reagents for grouping streptococci. J Clin Microbiol 1979; 10: 641-646.
59. Slifkin M, Engwall C, Pouchet GR. Direct-plate serological grouping of Beta-hemolytic streptococci from primary isolation plates with the Phadebact streptococcus test. J Clin Microbiol 1978; 8: 356-360.
60. Ainsworth AJ, Capley G. Monoclonal antibodies produced to *Streptococcus agalactiae*. Am J Vet Res 1986; 47: 1211-1213.
61. Jordan DC. The effect of quality and component premiums on mastitis awareness. Proc 24th Annu Meet Natl Mastitis Counc 1985: 86-91.
62. Politis I, Ng-Kwai-Hang KF. Effects of somatic cell count and milk composition on cheese composition and cheese making efficiency. J Dairy Sci 1988; 71: 1720-1727.
63. Oz HH, Hillmann DJ, Farnsworth RJ. Bulk tank milk analysis for isolating mastogenic bacteria. Dairy Food Sanit 1985; 5: 248-251.
64. Dodd FH. Symposium: Advances in understanding mastitis; Mastitis-progress in control. J Dairy Sci 1983; 66: 1773-1780.
65. Leslie KE, Dohoo IR, Meek AH. Somatic cell counts in bovine milk. Compend Contin Educ Pract Vet 1983; 11: s601-s610.
66. Sischo WM, Miller GY, Heider LE. Dry cow therapy and mastitis control, an observational study. Proc Am Assoc Bovine Pract 1992; 24: 179-180.
67. Erskine RJ, Eberhart RJ, Hutchinson LJ, Spencer SB. Herd management and prevalence of mastitis in dairy herds with high and low somatic cell counts. J Am Vet Med Assoc 1987; 190: 1411-1416.
68. Ward GE, Schultz LH. Relationship of somatic cells in quarter milk to type of bacteria and production. J Dairy Sci 1972; 55: 1428-1431.
69. Wanasinghe DD, Frost AJ. The prevalence of udder infection and mastitis in herds producing bulk milk with either consistently high or low cell count. Aust Vet J 1979; 55: 374-380.
70. Erskine RJ, Eberhart RJ. Herd benefit-to-cost ratio and effects of a bovine mastitis control program the includes blitz treatment of *Streptococcus agalactiae*. J Am Vet Med Assoc 1990; 196: 1230-1235.
71. Pankey JW. Premilking udder hygiene. J Dairy Sci 1989; 72: 1308-1312.
72. Parr BH. The *Strep. agalactiae* herd: An alternate approach. Proc Am Assoc Bovine Pract 1987; 19: 72-73.
73. Pankey JW. Hygiene at milking time in the prevention of bovine mastitis. Br Vet J 1989; 145: 401-409.
74. Thorburn MA. The use of the log-linear model to evaluate herd factors as determinants of *Staphylococcus aureus* and *Streptococcus agalactiae* mastitis occurrence in California dairy herds in 1977. 3rd Int Sym Vet Epidemiol Econ, Arlington, Virginia, 1982: 76-83.
75. Smith AR, Ward GE. Evaluation of methods for control of *Streptococcus agalactiae* in dairy herds. Can Vet J 1975; 16: 109-113.
76. Kingwill RG, Neave FK, Dodd FH, Griffin TK, Westgarth DR. The effect of a mastitis control system on levels of subclinical and clinical mastitis in two years. Vet Rec 1970; 87: 94-102.
77. Huber WG. Antibacterial drug effectiveness against mastitis pathogens. J Am Vet Med Assoc 1977; 170: 1182-1184.
78. Tyler JW. Treatment of subclinical mastitis. Vet Clin North Am Food Anim Pract 1992; 8: 17-28.
79. Erskine RJ. Therapy of clinical mastitis: Successes and failures. Proc 30th Annu Meet Natl Mastitis Counc 1991; 30: 40-49.
80. Philpot WN. Control of mastitis by hygiene and therapy. J Dairy Sci 1979; 62: 168-176.
81. Brown MB, Scasserra AE. Antimicrobial resistance in streptococcal species isolated from bovine mammary glands. Am J Vet Res 1990; 51: 2015-2020.
82. McDonald JS, McDonald TJ, Stark DR. Antibigrams of streptococci isolated from bovine intramammary infections. Am J Vet Res 1976; 37: 1185-1187.
83. Matthews KP, Oliver SP, Jayarao BM. Susceptibility of staphylococci and streptococci isolated from bovine milk to antibiotics. Agri-Practice 1992; 13: 18-24.
84. Messier S, Nadeau M, Higgins R. Sensibilité de *Streptococcus agalactiae* envers différents antibiotiques. Med Vet Quebec 1994; 24: 70-72.
85. Craven N. Efficacy and financial value of antibiotic treatment of bovine clinical mastitis during lactation — A review. Br Vet J 1987; 143: 410-422.
86. Thomson JR, Mollison N, Matthews KR. An investigation of mastitis due to *S. agalactiae*, *S. uberis* and *M. smegmatis* in a dairy herd. Vet Rec 1988; 122: 271-274.
87. Weaver LD, Galland J, Martin PAJ, Versteeg J. Treatment of *Streptococcus agalactiae* mastitis in dairy cows: Comparative efficacies of two antibiotic preparations and factors associated with successful treatment. J Am Vet Med Assoc 1986; 189: 666-669.
88. Batra TR. Effect of complete dry cow treatment on mastitis control in dairy cattle. Can J Anim Sci 1988; 68: 553-556.
89. Natzke RP, Everett RW, Bray DR. Effect of drying off practices on mastitis infection. J Dairy Sci 1975; 58: 1828-1832.
90. Francis PG. Update on mastitis III. Mastitis therapy. Br Vet J 1989; 145: 302-311.
91. Ahl AS, Gibson CD, Kirk JH, Kaneene JB, Ahl JG. Cost of mastitis and its prevention in four dairy cattle herds on St. Croix, U.S. Virgin Islands. J Am Vet Med Assoc 1989; 194: 1418-1421.
92. Edmondson PW. An economic justification of "blitz" therapy to eradicate *Streptococcus agalactiae* from a dairy herd. Vet Rec 1989; 125: 591-593.
93. Bar-Moshe B, Weiss I, Abuhanna F, Hativ N, Shine Y. A regional program for the eradication of *Streptococcus agalactiae* in Israeli dairy herds. Isr J Vet Med 1987; 43: 236-241.
94. Agger JF, Priou C, Huda A, Aagaard K. Risk factors for transmission of *Streptococcus agalactiae* infection between Danish dairy herds: A case control study. Vet Res 1994; 25: 234-238.